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Estimation of Growth and Photosynthetic Performance of Two C₄ Species (*Pennisetum spicatum* (L.) Körn. and *Zea mays* L.) under a Low Temperature Treatment

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ABSTRACT

Pearl millet (*Pennisetum spicatum* (L.) Körn.) and maize (*Zea mays* L.) are C₄ grass species grown for feeding humans and animals in Almadinah Almunawwarah, which is in the western part of Saudi Arabia. During the winter, the mean temperature, which drops to 14°C, represents a major problem for the growth of these species in this region. Therefore, the objectives of this research were to investigate the growth response and the photosynthetic performance of *P. spicatum* and *Z. mays* under a low temperature stress. The treatments involved daytime and nighttime temperatures of 14/12°C (low temperature) and 24/22°C (optimum temperature). The results indicated that low temperature significantly reduced all growth and physiological parameters, including seed germination, leaf expansion, leaf area, shoot length and root length of the two species compared to those of the control. Additionally, the low temperature significantly decreased the light-saturated assimilation rate (A_{sat}), quantum yield (ϕ), saturated rate of carbon dioxide uptake (A_{max}) and efficiency of carboxylation on both species compared to those of the control. Moreover, the values of F_v/F_m and the chlorophyll contents of both species were significantly reduced by low temperature compared to those of the control. It can be concluded that both species had little tolerance to low temperatures.

KEYWORDS

Pennisetum spicatum; *Zea mays*; low temperature; plant growth; CO₂ uptake; photochemistry

1 Introduction

One of the most critical variables that restricts plant development, distribution and production is low temperature [1–3]. The majority of C₄ plants are mainly restricted to regions with relatively warm temperatures because low temperatures adversely influence their physiological processes [2,4]. Sowinski et al. [5] observed that the rates of both leaf cell division and elongation of C₄ plants were lower at low than warm temperatures. All stages of plant growth, including germination and early seedling growth, and a wide range of physiological processes are also considered to be influenced by low temperatures [3,6–8]. Several studies have shown that plants have a rapid and sensitive growth response to low temperatures [3,5,8–10]. *Z. mays* is the world's most grown C₄ species [3]. Exposure of *Z. mays* to low temperatures impairs leaf growth and photosynthesis and breaks down chlorophyll in developed leaves [11]. The leaf expansion rate was found to be the major factor determining the variations in leaf growth



rates of nine varieties of sugar beet at four different temperature treatments [12]. This indicates that low temperature is one of the most important factors influencing C₄ plant growth and development.

Under optimum environmental conditions, the efficiency of photosynthesis is 40% greater in C₄ than C₃ plants [4]. This benefit, however, is not obtained under low temperature conditions in C₄ plants. Low temperatures impair thylakoid membranes, reduce chlorophyll contents and decrease the photosynthetic potential of C₄ crops such as *Z. mays* [5,13]. The decrease in photosynthesis ability of leaves of *Z. mays* under cold temperatures is related to decreases in both the light-saturated assimilation rate (A_{sat}) and the maximum quantum yield (ϕ) [6,8,14]. On the other hand, some C₄ crop species are acclimated to and grow in cold climates [15]. The ability of these crop plants to stimulate their physiological processes and photosynthesis or to increase them under cold temperatures allows them to live in these relatively cool environments [5,9,16].

Photoinhibition can occur during photosynthesis when light absorption exceeds that needed by the photosynthetic demands of plants [17]. Several studies have revealed that plants experience low temperature photoinhibition, both under controlled conditions and in the field [1,6,18]. Photoinhibition is described by a decrease in the quantum yield of CO₂ absorption (ϕ) and the ratio between the variable and maximum fluorescence of chlorophyll a (F_v/F_m) [1,19]. PSII photoinhibition is demonstrated by a decrease in the F_v/F_m value of dark-adapted leaves. Furthermore, the reduction rate depends on environmental factors sooner than a photo-inhibitory treatment and genotypic variability [5–6,20].

Pearl millet (*Pennisetum spicatum*) and Maize (*Zea mays*) are C₄ grass species grown for feeding humans and animals in Almadinah Almunawwarah, which is located in the western part of Saudi Arabia. During the winter, the mean temperature, which drops to 14°C, represents a major problem for the growth and photosynthesis of these species in this region (Presidency of Meteorology and Environment, Saudi Arabia; <https://ncm.gov.sa/Ar/Weather/Region/Weather/Pages/Madinah.aspx>). Since there is a lack of information concerning the cold tolerance of *P. spicatum*, this work was performed to investigate the growth response and photosynthetic performance of the *P. spicatum* grass at low temperatures. The treatments involved daytime/nighttime temperatures of 14/12°C (low temperature) and 24/22°C (optimum temperature). All growth factors and the photosynthetic performance were compared to those of *Z. mays*.

2 Materials and Methods

2.1 Seed Germination

Seeds of commercial pearl millet (*Pennisetum spicatum* L. cv. Madinah) and maize (*Zea mays* L. cv. Legacy SU; USA) were obtained from Al-Hilali Agricultural Company, Almadinah Almunawwarah, Saudi Arabia. The experiments in this research were carried out in the Biology Department, Science Faculty, University of Taibah, Almadinah Almunawwarah, Saudi Arabia. Seeds of both species were surface sterilized by soaking them for ten minutes in 0.1% mercury chloride. After five washes in sterile distilled water, seeds were placed on two layers of Whatman No. 1 filter paper inside a Petri dish (9 cm × 1.6 cm) and watered with 10 ml of distilled water. Each Petri dish contained 10 seeds, and dishes containing four replicates of each species were placed in an incubator in darkness at two different temperatures (14 and 24°C); there was a total of 16 Petri dishes. The appearance of a radicle from the seeds was considered a sign of germination. The germination process was documented every day for the whole experimental phase, which lasted for one week.

2.2 Plant Growth Parameters

P. spicatum and *Z. mays* seeds were grown in 12 cm × 20 cm plastic pots containing 2 kg of compost. Pots were located inside an environmentally regulated chamber (JSR 314-240, Inc., JS Research., 40-1 Gumsang-Dong, Gongju Area, Korea) with a light/dark photoperiod of 14/10 h and 60% relative humidity. For lighting, fluorescent and halogen lamps were used, resulting in a total of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$

irradiance. The treatments involved daytime/nighttime temperatures of 14/12°C (low temperature) and 24/22°C (optimum temperature). Four replicates for each species were used at each temperature treatment, resulting in a total of 16 pots. Five seeds were planted in each pot, and the seedlings were thinned after emergence, leaving two per pot. The plants were irrigated to field capacity with a full-strength Hoagland solution. The following growth parameters were measured at the end of the experiment (60 days after planting): plant height, leaf number, and root and shoot fresh weight. The area of leaves was measured by a LI-COR leaf area meter (LI-COR Inc., Lincoln, Nebraska, USA). The shoot and root samples were dried in an oven for 48 h at 80°C to estimate dry weight.

2.3 Gas Exchange Measurements

Rates of gas exchange of mature fully expanded leaves of *P. spicatum* and *Z. mays* were calculated using a LI-COR 6400XT infrared gas analyser (LI-6400, LI-COR Inc., Lincoln, Nebraska, USA). For the measurements of photosynthesis and dark respiration, the fourth youngest fully expanded leaf was used. Light response curves were generated at a 24°C leaf temperature and 410 $\mu\text{mol mol}^{-1}$ (C_a) atmospheric CO_2 concentration. The leaves were steadily illuminated with photon flux densities between 0 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The Von Caemmerer and Farquar equations were used to calculate the net photosynthesis per unit leaf area and intercellular CO_2 concentration c_i [21]. A_{sat} was measured at saturating photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at ambient CO_2 concentration of 410 $\mu\text{mol mol}^{-1}$. CO_2 response curves (A/C_i) within the 50–550 $\mu\text{mol mol}^{-1}$ range were generated using PPFD of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at a 24°C leaf temperature, as defined by Collatz et al. [22].

2.4 Chlorophyll Content Determination

The chlorophyll content was measured and expressed as the chlorophyll content index using a handheld chlorophyll content meter (CCM-200, Opti-Sciences, USA). In the different treatments, the fourth newest completely expanded leaf was used to calculate the chlorophyll content of each species four times, and the average was used for analysis.

2.5 Chlorophyll Fluorescence Measurements

A portable fluorimeter was used to measure the chlorophyll fluorescence of the fourth newest completely expanded leaf of both *P. spicatum* and *Z. mays* (PEA, Hansatech, Norfolk, Lynn Kings). The leaves were dark acclimated for 20 min before the fluorescence was measured. As described by Al-shoaibi [23], the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) was measured four times for *P. spicatum* and *Z. mays* species.

2.6 Statistical Analysis

The data obtained from the different measurements were analysed statistically using one-way and two-way analysis of variance (ANOVA) and with a general linear model for evaluating the primary effects and interactions of the factors investigated (i.e., species and temperature). The significance of the different levels of the variables analysed was tested, and multiple comparisons were carried out using the Tukey test. All analyses were performed using version 15 of Minitab (Brandon Court, Progress Way Unit E1-E2, CV3 2TE, Coventry, Great Britain). Four replicates were used for each temperature treatment, and standard deviations and standard errors were calculated using Microsoft Excel 2016.

3 Results

3.1 Seed Germination

The effects of the two temperature treatments on the seed germination percentages of *P. spicatum* and *Z. mays* are illustrated in Fig. 1. At 24°C, seed germination of *P. spicatum* started on the first day and was

completed on the third day, while seed germination of *Z. mays* started on the second day and was completed on the fourth day of the germination trial. On the other hand, at 14°C, seed germination of *P. spicatum* started on the first day and was completed on the third day, while seed germination of *Z. mays* started on the second day and was completed on the fifth day of the germination trial. The final germination percentages for both species approached 100% in the two temperature treatments. Thus, temperature had no apparent significant effect on the final germination percentages attained by seeds of both species. However, the germination rate for *Z. mays* seeds at 14°C significantly decreased compared to that of *Z. mays* seeds germinated at 24°C ($p < 0.01$).

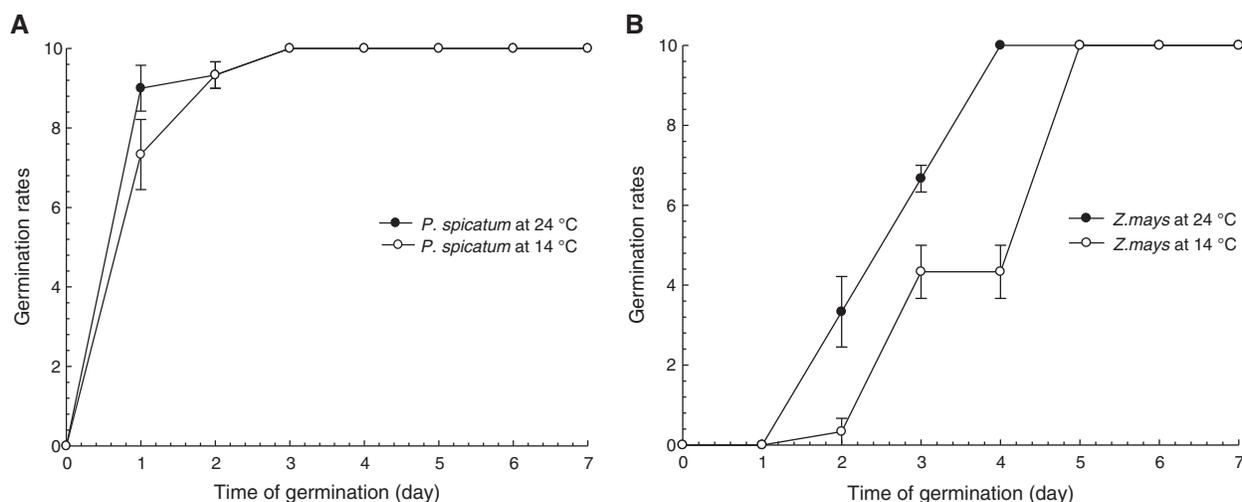


Figure 1: Effects of temperature treatments (14°C and 24°C) on seed germination of the two C_4 species; *P. spicatum* (A), and *Z. mays* (B); ($n = 4$, Mean \pm S.E.)

3.2 Plant Growth Parameters

A number of growth parameters of both *P. spicatum* and *Z. mays* were measured to determine how they were affected by low-temperature treatment; the results are summarized in Tab. 1. Low temperature significantly affected all growth parameters of the two species ($p < 0.001$). Moreover, the results showed that all growth parameters of *P. spicatum* and *Z. mays* decreased significantly when the plants were grown at 14°C compared to 24°C ($p < 0.001$; Fig. S1). Between both species, *P. spicatum* showed the highest percentage of reduction in all growth parameters at 24°C ($p < 0.001$; Tab. 1). The fresh weight and dry weight of roots of *P. spicatum* grown at 14°C were 99% and 98.33% lower than those of the same species grown at 24°C; these decreases were the highest observed among these indices. Similarly, the leaf area and dry weight of shoots of *Z. mays* grown at 14°C were 93.77% and 91.42% lower than those of the same species grown at 24°C ($p < 0.001$; Tab. 1), representing the largest percent decreases for these indices.

Table 1: Effects of temperatures on the growth parameters of *P. spicatum* and *Z. mays* ($n = 4$, Mean \pm S.E.). Means that do not share a letter are significantly different at $p \leq 0.05$

Trait	Species	14°C	24°C
Plant height (cm)	<i>P. spicatum</i>	16.67 ^d \pm 0.88	49 ^b \pm 2.65
	<i>Z. mays</i>	29.33 ^c \pm 5.36	63.67 ^a \pm 0.88
Leaf number	<i>P. spicatum</i>	3 ^d \pm 0.0	7 ^a \pm 0.0
	<i>Z. mays</i>	3.67 ^c \pm 0.33	6 ^b \pm 0.0
Leaf area (cm ²)	<i>P. spicatum</i>	10.2 ^d \pm 0.3	343 ^b \pm 14
	<i>Z. mays</i>	58 ^c \pm 8.71	932.4 ^a \pm 24
Fresh weight of the whole shoot (g)	<i>P. spicatum</i>	0.7 ^c \pm 0.06	8.67 ^b \pm 0.73
	<i>Z. mays</i>	1.4 ^c \pm 0.4	15.96 ^a \pm 0.62
Dry weight of the whole shoot (g)	<i>P. spicatum</i>	0.05 ^c \pm 0.02	0.8 ^b \pm 0.05
	<i>Z. mays</i>	0.12 ^c \pm 0.01	1.4 ^a \pm 0.15
Root length (cm)	<i>P. spicatum</i>	9.67 ^c \pm 2.3	31.67 ^b \pm 3.84
	<i>Z. mays</i>	25.66 ^b \pm 2.34	50.33 ^a \pm 6.84
Fresh weight of root (g)	<i>P. spicatum</i>	0.05 ^b \pm 0.001	5.16 ^a \pm 0.75
	<i>Z. mays</i>	0.64 ^b \pm 0.03	4.34 ^a \pm 0.49
Dry weight of root (g)	<i>P. spicatum</i>	0.005 ^b \pm 0.0	0.3 ^a \pm 0.0
	<i>Z. mays</i>	0.06 ^b \pm 0.005	0.36 ^a \pm 0.03

3.3 Gas Exchange Measurements

The results presented in Fig. 2 illustrate the photosynthetic CO₂ (A) absorption in response to photon flux (Q) for *P. spicatum* and *Z. mays* grown in the two temperature treatments. The temperature treatments significantly affected the photosynthetic performance of the two species ($p < 0.001$). The highest photosynthesis efficiency was observed at 24°C for both species (Fig. 2). The rates of light saturation (A_{sat}) for *P. spicatum* and *Z. mays* grown at 14°C were considerably lower than those of the same species grown at 24°C ($p < 0.01$; Fig. 3A), with percent decreases of 62.8% and 19.7% for *P. spicatum* and *Z. mays*, respectively. Additionally, the quantum yields (ϕ) for *P. spicatum* and *Z. mays* grown at 14°C were considerably lower than those of the same species grown at 24°C ($p < 0.01$; Fig. 3B), with percent decreases of 51.9% and 17.9% for *P. spicatum* and *Z. mays*, respectively. Additionally, Fig. 3C shows the measurements of the A/c_i curve for *P. spicatum* and *Z. mays* under the two temperature treatments. Generally, the A/c_i curve plateaus (A_{max}) and the carboxylation efficiency of *P. spicatum* and *Z. mays* were considerably lower when plants were grown at 14°C compared with 24°C ($p < 0.01$; Figs. 3C, 3D). For both species, the highest percent decreases in A_{max} and carboxylation efficiency were observed for *P. spicatum* grown at 14°C; these were 62.8% and 67.4% lower than those of the same species grown at 24°C ($p < 0.01$; Figs. 3C, 3D). Additionally, all the photosynthetic parameters of *P. spicatum* grown at 14°C were significantly lower than those of *Z. mays* grown at the same temperature (Fig. 3).

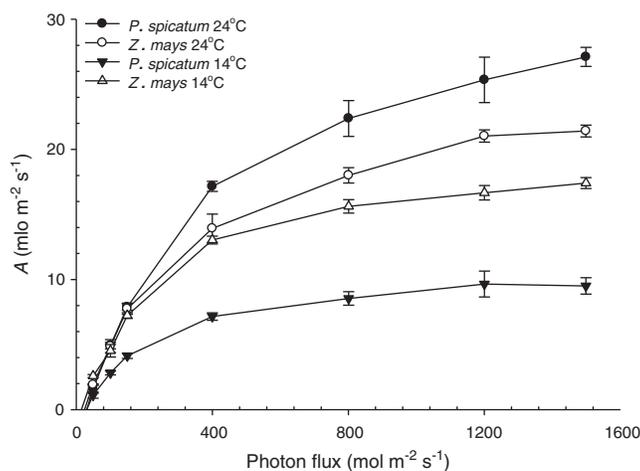


Figure 2: The photosynthetic CO₂ absorption response (A) per unit area of the leaf to photon flux (Q) for the two C₄ species, *P. spicatum* and *Z. mays* ($n = 4$, Mean \pm S.E.). CO₂ absorption measurements were all made at 24°C and C_a of 410 $\mu\text{mol mol}^{-1}$

3.4 Chlorophyll Content and Chlorophyll Fluorescence

Fig. 4A illustrates the effects of the two temperature treatments on the chlorophyll content of *P. spicatum* and *Z. mays*. Compared to the chlorophyll contents of the same species grown at 24°C, the chlorophyll contents of *P. spicatum* significantly decreased in response to the decreased temperature (14°C) ($p < 0.01$; Fig. 4A). However, the chlorophyll contents of *Z. mays* grown at 14°C did not significantly decrease compared to those of the same species grown at 24°C (Fig. 4A). The maximum quantum efficiency of PSII (F_v/F_m) photochemistry of *P. spicatum* and *Z. mays* grown under the two temperature treatments is shown in Fig. 4B. For both species, the chlorophyll fluorescence values of the species grown at 14°C significantly decreased compared to the values of the two species grown at 24°C ($p < 0.01$; Fig. 4B). Additionally, the F_v/F_m value of *P. spicatum* grown at 14°C was significantly greater than the F_v/F_m value of *Z. mays* grown at the same temperature ($p < 0.05$; Fig. 4B).

4 Discussion

One of the most important factors affecting photosynthetic efficiency and plant growth is temperature [24,25]. This research investigated the photosynthesis gas exchange and growth responses of *P. spicatum* and *Z. mays* to low temperature. The results showed that all seeds of both *P. spicatum* and *Z. mays* were able to complete germination under the two temperature treatments (Fig. 1). The ability of seeds to germinate over a wide range of temperatures is important for plant establishment in many different environments [26]. The results obtained showed that the optimum temperature for quick seed germination for both species was 24°C. Similar findings have been reported for seed germination of two pearl millet cultivars [27]. In addition, compared with *Z. mays*, *P. spicatum* presented a faster rate of germination under low temperature.

A substantial environmental variable that governs the development and growth of plants is temperature [24,28]. For both *P. spicatum* and *Z. mays* used in this study, all the growth parameters were significantly lower when the plants were grown at 14°C than when they were grown at 24°C (Tab. 1). The optimum temperature for the growth of seedlings of both species was 24°C. It was previously reported that the optimum temperature for *Z. mays* seedling growth ranged from 25°C to 28°C [2]. Compared with *P. spicatum*, *Z. mays* grown at 14°C grew better overall, especially in terms of leaf area and root length (Tab. 1). Muhl et al. [29] found that a considerably greater leaf area of *Moringa oleifera*

increased the whole number of stomata per plant, which resulted in increased vegetative growth and dry matter accumulation. Thus, compared with *P. spicatum*, *Z. mays* grown at 14°C presented higher percent increases in fresh and dry weights of both the shoots and roots grown at the same temperature. The differences found can be explained by genetic variations between the two species examined [30].

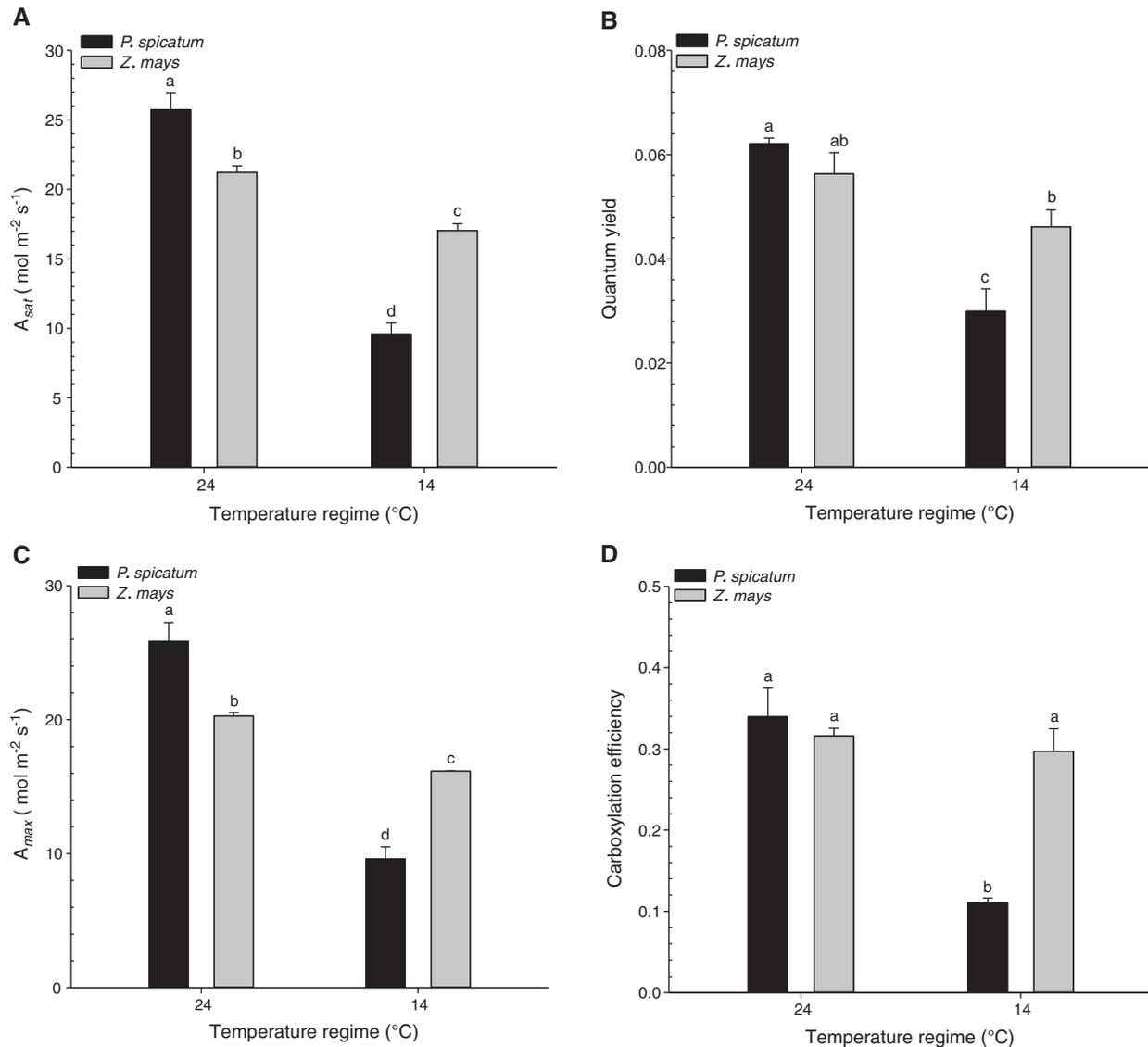


Figure 3: Effects of temperature treatments (14°C, and 24°C) on physiological parameters of the two C_4 species; *P. spicatum* and *Z. mays* ($n = 4$, Mean \pm S.E.). (A) A_{sat} , (B) Quantum yield, (C) A_{max} and (D) Carboxylation efficiency. Means that do not share the same letter are significantly different at $p \leq 0.05$

In addition, the gas exchange parameters of *P. spicatum* and *Z. mays* in this study were significantly influenced by low temperature. The photosynthetic measurements showed that the optimal temperature for gas exchange of the two species was 24°C (Fig. 3). Additionally, both *P. spicatum* and *Z. mays* exhibited nearly similar quantum yields and carboxylation efficiencies at 24°C. For the leaves of all plants grown at 24°C, the photosynthetic rates of both *P. spicatum* and *Z. mays* were comparable and similar to previously documented values for healthy leaves from a variety of NADP-malic enzyme-type

C₄ grass species [31]. This shows that these two species were unstressed and not experiencing photoinhibition at the optimum temperatures and exhibited relatively high F_v/F_m . However, the 14°C temperature caused a significant decrease in A_{sat} and A_{max} in *Z. mays*, and a substantial decrease in A_{sat} , ϕ , A_{max} and carboxylation efficiency on *P. spicatum* compared with those of plants grown at 24°C. The photosynthesis inhibition of the two species grown at 14°C may be partly due to a decrease in stomatal conductance or a reduction in photosynthetic pigment content. All of these factors influence the activities of photosystem II (PSII), clearly leading to the deactivation of enzymes that play a key role in photosynthesis [32,33]. In both controlled environments and the field, a reduction in the photosynthetic efficiency of C₄ species has previously been observed at low temperatures [2–3,8,33]. The decrease in photosynthesis of these two species could be the result of a reduction in the activity of both phosphoenolpyruvate carboxylase (PEPC) and pyruvate dikinase (PPDK) enzymes [22,34]. Other reasons for the decrease in the photosynthesis might be limitations in the transfer of electrons from the thylakoid membrane or decreases in ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity [35,36].

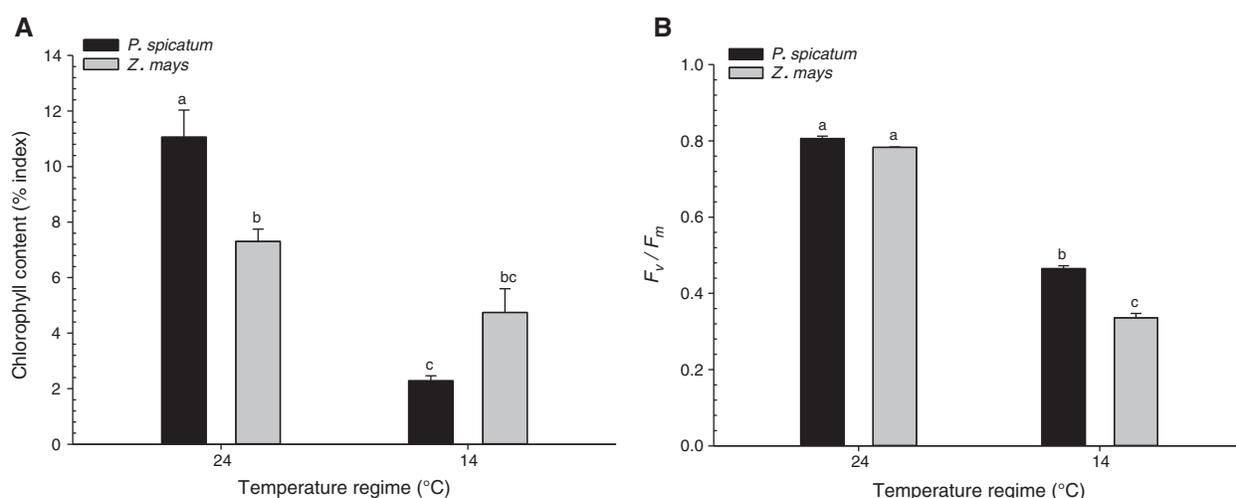


Figure 4: Effects of temperature treatments (14°C, and 24°C) on physiological parameters of the two C₄ species, *P. spicatum* and *Z. mays* ($n=4$, Mean \pm S.E.). (A) Chlorophyll content and (B) F_v/F_m . Means that do not share the same letter are significantly different at $p \leq 0.05$

In many crop species that have different responses to low temperatures, a decrease in chlorophyll content has been identified [23,37,38]. Similarly, the findings in Fig. 4A show a decrease in the chlorophyll content of *P. spicatum* at low temperatures. This decrease may be caused by chlorophyll degeneration at this temperature [38]. Another possibility for this decrease in chlorophyll content may be due to the irregular development of chloroplasts [5,37]. On the other hand, the outcomes of this study provide clear proof that both *P. spicatum* and *Z. mays* showed significantly lower resistance to photoinhibition at low temperature. The low F_v/F_m values of both species grown at low temperature may be due to impaired development of the photosynthetic apparatus. Nie et al. [39] demonstrated that some polypeptides of chloroplasts were poorly expressed in *Z. mays* leaves that developed at 14°C. The abundance of these proteins, including the D1 protein, might account for the low values of F_v/F_m , even during the lack of direct photoinhibition.

5 Conclusion

This study investigated the growth response of *P. spicatum* and *Z. mays* by estimating the photosynthetic performance under low temperature stress. Low temperature significantly reduced all growth and

physiological parameters, including seed germination, leaf expansion, leaf area, shoot length and root length, of the two species compared to those of the control plants. Moreover, the photosynthetic performance, the values of F_v/F_m and the chlorophyll contents of both species were significantly reduced by low temperature compared to those of the control. It can be summarize that both species had little tolerance to low temperatures.

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Conflicts of Interest: The author declares that he has no conflicts of interest to report regarding the present study.

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Appendix



Figure S1: Pearl millet (*Pennisetum spicatum* (L.) Körn.) and maize (*Zea mays* L.) grown at temperature treatments of 24°C and 14°C